

DNA Binding and Bending by the Integration Host Factor (IHF)

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Integration Host Factor is a sequence specific DNA binding protein known to recognize several sites in the *E. Coli* and lambda phage genomes. In site-specific recombination of the lambda phage genome with that of the *E. Coli* host, IHF serves as an architectural protein shown to be indispensable to the formation of a nucleoprotein complex that precedes the recombination event. Regardless of considerable differences in size between the IHF binding sites from the lambda phage genome, circular permutation gel electrophoresis data show that IHF bends the DNA by greater than 140 degrees at each site. The crystal structure of the H' site bound to IHF shows the DNA bent into a U-turn and wrapped around the protein. The structure identifies two positions in the DNA substrate where proline side chains, one from each sub-unit of IHF, intercalate between AT base pairs and induce kinks in the helix. Synchrotron x-ray footprinting analysis of IHF binding and bending has been initiated with exploratory kinetic measurements using the complementary DNase I kinetics assay developed in the Brenowitz laboratory. Preliminary equilibrium titrations have established the nM affinity of IHF for the reference-binding site. Preliminary kinetics results suggest that binding does not follow simple 2nd order concentration dependence in protein. Synchrotron studies will determine whether the reaction profile includes a detectable intermediate in which the protein is bound, but the DNA is not yet bent. If this is the case, we should be able to characterize the dynamics of the bending process, including its dependence on environmental variables.